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Comm.
Dr. Gardner
Dr. Huebner
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CANCER

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

JUL 31 1973

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☒ Second Renewal ☐ Date: July 31, 1973

1. Principal Investigator (give title and degrees):

Harry L. Ioachim, M.D.
Professor Adjunct of Pathology
College of Physicians and Surgeons
of Columbia University.

2. Institution & address:

Lenox Hill Hospital
Department of Pathology
100 East 77th Street
New York, N. Y. 10021

3. Department(s) where research will be done or collaboration provided:

Pathology

4. Short title of study:

Studies of Human Lung and Ovarian Carcinomas

5. Proposed renewal date: July 31, 1974

6. How results to date have changed earlier specific research aims:

During the first year of this grant the immunological studies of tumor-associated antigens in cancers of ovary and lung, have progressed satisfactorily resulting in several findings of interest. To follow and expand these results, major emphasis will be given in this second year to the immunological studies.

The tissue culture and electron microscopy studies, testing possible squamous metaplasia in vitro under exposure to smoke will be continued.

7. How results to date have changed earlier working hypothesis:

No significant changes in working hypotheses were made.

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8. Any additional facilities now required? Describe briefly:

Scintillation counters for beta and gamma radiations were needed, in order to explore cellular immunity in lung and ovarian carcinomas. Both scintillation counters were acquired with funds provided expressly for this research by an outside donor.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

Dr. Sheldon C. Sommers has expressed the desire to act as consultant for this research project rather than principal investigator, due to limitations of time.

Dr. John A. Terzakis previously part of this project will also act as associate principal investigator.

Dr. Margaret E. Long has retired from full time activity in the Department of Pathology and will collaborate in the future as consultant with our team.

Ana Walderramus who has resigned will be replaced with Edward Paluch who has previously worked for this project on a voluntary basis.

Tove Bamberger would be a part time secretary for this grant, in charge with bookkeeping, ordering, reference filing and manuscripts writing.

10. Append outline of experimental protocol for ensuing year. - see attached protocol

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

1. - Common Antigenic Component in Ovarian Carcinoma: Demonstration by double Diffusion and Immunofluorescence Techniques - B. H. Dorsett and H. L. Ioachim - Immunological Communications, 2: 173-184, 1973
2. Antigenic and Morphologic Properties of Ovarian Carcinoma -H. L. Ioachim. B. H. Dorsett, M. Sabbath, B. Andersson and H. R. K. Barber- Gynecologic Oncology, 1:000, 1973.
3. Antigenic Component Associated with Human Ovarian Carcinoma- B. H. Dorsett, M. Sabbath and H. L. Ioachim- Feder. Proc. pp. 4442, 1973.
4. Neurosecretory Appearing Cells of Human Segmental Bronchi-J. A. Terzakis Sheldon C. Sommers and B Andersson - Lab. Investigation 26:127, 1972
5. Ultrastructure of human endometrial adenocarcinoma-J. A. Terzakis, S. C. Sommers and B. Andersson-(in progress).
6. Age Changes in Ultrastructure and Histochemistry of Human Bronchi- M. A. Long, B. Andersson, J. A. Terzakis and S. C. Sommers (in preparation)

12. Summary progress report (append in standard form as separate document, unless recently submitted).

see attached report

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13. Budget for the coming year:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Sheldon C. Sommers M.D. - Consultant	10%	none
Harry L. Ioachim M.D. - Principal Investigator	25%	none
John A. Terzakis M.D. - Associated Principal Investigator	25%	9,000
Margaret E. Long Ph.D. - Consultant	10%	2,500
Elena C. Schmidt Ph.D. - Investigator	50%	10,000
Brent H. Dorsett B.S. - Investigator	100%	11,500
Technical		
Marjorie Rosenberg B.S.	100%	10,000
Antonia Pearse B.S.	30%	3,000
Edward Paluch	30%	3,000
Tove Bamberger - Secretary	30%	3,000
	Sub-Total for A	52,000
B. Consumable supplies (by major categories)		
Reagents (immunology)		1,000
Tissue culture media		1,000
Glassware, isotopes, photomaterial		1,500
Animals and animal food		1,000
Electron Microscopy (supplies and services)		6,000
	Sub-Total for B	10,500
C. Other expenses (itemize)		
Travel		1,600
Publication costs, illustrations, mailing, miscellaneous		2,000
	Sub-Total for C	3,600
	Running Total of A + B + C	66,100
D. Permanent equipment (itemize)		
1. Swinging Bucket Rotor Type SW 41 Ti for ultracentrifuge		2,750
2. Electrophoresis apparatus		650
3. Immunoelectrophoresis unit		400
	Sub-Total for D	3,800
E. Indirect costs (15% of A+B+C)	E	9,915
	Total request	79,815

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14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
None			

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
None			

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Harry L. Ioachim, M.D.

Signature [signature] Date July 31, 1973

Telephone 212 (Area Code) 794-4420 (Number) (Extension)

Checks payable to

Lenox Hill Hospital

Mailing address for check:

100 East 77 Street
New York, N.Y. 10021

Responsible officer of institution

Typed Name Louis F. Parker

Title Vice President

Signature [signature] Date 7/31/73

Telephone 212 (Area Code) 794 (Number) 5141 (Extension)

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ADDENDUM

10. Outline of Experimental Protocol for Ensuing Year

A. Tumor-associated antigens of lung tumors.

Bronchial squamous cell carcinoma representing over 50% of all lung cancers is the histologic type generally believed to be associated with smoking and air pollution. Squamous cells, normal and neoplastic, contain numerous doubly refractive tonofibrils forming a tridimensional framework around the nucleus. The tonofibrils contain sulfhydril and disulfide groups and are assumed to be the precursors of keratin. When keratin, a poorly soluble substance, comes into direct contact with mezenchymal tissues, a granulomatous inflammation is elicited, characterized by the accumulation and proliferation of reticulo-endothelial cells. A crucial point in these types of reactions is to differentiate between granulomatous hypersensitivity and response to a colloidal foreign body substance. In our preliminary studies the local reaction to keratin was accompanied by a humoral response in which sera of animals injected with keratin reacted specifically with keratinized squamous epithelial cells. As part of our study of tumor-associated antigens it was decided to analyze this reaction to keratin in order to apply it subsequently to the detection of keratin-forming squamous cell carcinomas.

At a later stage radioactive labeling of gammaglobulins will be attempted and their attachment to squamous cells of the epidermis as well as to squamous cell carcinoma followed by immunofluorescence and electron microscopy. At the same time, sera of patients with squamous cell carcinoma will be tested for reactivity against squamous cells and extracts thereof.

B. Tumor-associated antigens of ovarian carcinoma.

Purification of the antigenic component found in ovarian carcinoma will be undertaken with the use of gel filtration and ion exchange chromatography, alongwith preparative ultracentrifugation. The highly purified antigen thus obtained will be used to produce monospecific antisera by heterologous immunization. It is to be assumed that ovarian carcinoma antigens in the circulating blood of patients will be in almost infinitesimal amounts, therefore making the availability of very sensitive

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techniques a prerequisite for their detection. The production of highly purified antigen preparations and antisera will be necessary for the development of such sensitive techniques. Radioimmunoassay procedures as the most sensitive techniques presently in use, will be developed for the search of ovarian carcinoma antigens in the peripheral blood of tumor patients. In addition , the purified antigenic preparations will be used to test the cellular immune system of these patients. For this purpose we are presently adapting the macrophage migration inhibition test and the lymphoblast transformation assay using our new liquid scintillation counter.

C. Squamous metaplasia induced by smoke in cultures of epithelial tumors.

The cultures subjected to the effects of smoke during the first part of this study will now be processed for light and electron microscopy and studied for possible cytologic changes. Keratin-forming squamous epithelium will be grafted subcutaneously in rats and guinea-pigs and the grafts will be removed at various intervals from 1 hour to 30 days. The tissue reaction to the graft will be studied in light and electron microscopy.

Extracts of keratinized squamous epithelium will be prepared and used to immunize rabbits according to methods previously described. Sera reacting with extracts of squamous epithelium in gel immuno-diffusion assays will be fractionated for isolation of gammaglobulins.

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ADDENDUM

12. Summary Progress Report.

A. Tumor-associated antigens of lung tumors.

The investigation of antigens associated with carcinomas of the lung began during the first year of this grant in accordance with a protocol similar to that used in the previous work on ovarian carcinoma. Heterologous sera obtained from rabbits immunized with lung tumor extracts were absorbed and tested in gel immunodiffusion and immunofluorescence assays. The results were not as specific and consistent as in the tests of ovarian carcinomas. New animals have been therefore immunized with better purified extracts and according to different schedules of immunization.

In addition, a new line of investigation has been developed. It is a common observation that squamous cell carcinomas freqently elicit granulomatous reactions due to their content of keratin. In the lung as well as in other locations, keratin, a poorly soluble substance produced by squamous cell carcinomas, induces an inflammatory process mediated through the accumulation of reticulo-endothelial cells, similar in many respects to a hypersensitivity reaction. The possibility that this reaction might be accompanied by general humoral or cellular events, which then could be used for its detection, stimulated our interest for its study.

The granulomatous inflammatory reaction was reproduced experimentally by the introduction of portions of keratinized epidermis in the subcutaneous tissue of rats and guinea pigs. The local tissue reaction was studied histologically at various intervals and the sera of these animals assayed against squamous epithelia in immunofluorescence tests. Positive specific immunofluorescent reactions were consistently obtained with sera of sensitized animals against keratin and keratohyalin-containing squamous cells, including components of squamous carcinomas.

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B. Tumor-associated antigens of ovarian carcinomas.

The need for immunological diagnosis in carcinoma of the ovary, a tumor that is inoperable in 60% of the patients at the time of clinical diagnosis, has been stressed repeatedly. During the first year of this grant, earlier studies on the culture in vitro and immunology of ovarian carcinoma have been continued and expanded. The ultrastructure of various types of ovarian tumors has been examined and a total of over 70 carcinomas of the ovary grown in tissue cultures for intervals of up to 10 months. A bank of lyophilized extracts of different types of ovarian tumors has been established in our laboratory. Heterologous antisera to pools of ovarian tumor extracts were raised in rabbits and then extensively absorbed with normal human tissues including normal ovaries, and serum components. These highly absorbed antisera assayed in gel immunodiffusion tests against a large variety of normal and neoplastic tissue extracts detect specifically and consistently the ovarian carcinoma extracts.

In indirect immunofluorescence assays both ovarian carcinoma tissue cultures and ovarian carcinoma cell suspensions, show specific cytoplasmic staining with the absorbed heterologous antisera in contrast to normal ovaries, benign ovarian tumors and unrelated malignant human tumors.

These results indicate the existence of a common, probably cross reacting, antigenic component shared by different ovarian carcinomas. The concentration, purification and characterization of this antigenic component is attempted at the present time.

The granulomatous inflammatory reaction was reproduced experimentally by the intradermal portions of formalinized epithelial

C. Squamous metaplasia induced by smoke in cultures of epithelial tissues

According to our proposal, cultures of various epithelial tissues, were submitted to periodical smoking using a standard CTR type smoking apparatus. The cultures used, included normal embryonal human lung and kidney, normal embryonal rat lung and kidney, normal human amnion, ovarian carcinoma, mammary carcinoma and lung adenocarcinoma. After the lethal toxic dose was established, smoking of these cultures was performed twice a week for up to four months. Coverslips of smoked cultures and matched controls were removed at similar periodical intervals, fixed, stained and examined under the light microscope. Similarly, smoked and control cultures were fixed and embedded for electron microscopy.

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